(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 June 2001 (21.06.2001)

PCT

(10) International Publication Number
WO 01/43876 A1

(51) International Patent Classification[7]: B01L 3/00, 3/02

(21) International Application Number: PCT/US00/34361

(22) International Filing Date:
18 December 2000 (18.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/465,959	17 December 1999 (17.12.1999)	US
09/465,960	17 December 1999 (17.12.1999)	US
09/466,314	17 December 1999 (17.12.1999)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US Not furnished (CIP)
Filed on Not furnished

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/43876 A1

(54) Title: DEVICES AND METHODS FOR MAKING BIOARRAYS

(57) Abstract: The present invention relates to a system for the fabrication of a bio-array and a method of dispensing of a controlled amount of liquid onto a surface. More particularly, this invention includes a bio-array fabricating system and a method for the controlled dispensing of small quantities of a reactive liquid within or onto a known target.

DEVICES AND METHODS FOR MAKING BIOARRAYS

This application is a continuing application of U.S.S.N.s 09/465,314, filed December 17, 1999; 09/465,960, filed December 17, 1999 and 09/465,959, filed December 17, 1999, all of which are expressly incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to a system for the fabrication of a bio-array and a method of dispensing of a controlled amount of liquid onto a surface. More particularly, this invention includes a bio-array fabricating system and a method for the controlled dispensing of small quantities of a reactive liquid within or onto a known target.

BACKGROUND OF THE INVENTION

Molecular biology is an ever expanding field of study. Of great importance within the field of molecular biology is the detection and analysis of DNA, RNA, bacteria, and proteins. Currently it is predicted that a large market exists for bio-chips (micro-array chips) in the diagnosing and treating of diseases. Envisioned is a day when physicians will have the capabilities to use bio-chips to make an immediate genomic marker based diagnosis in their offices without the need for a lab as an intermediate diagnostic facility. Currently, the greatest emphasis and market existence for bio-chips is within the field of genetic and pharmaceutical research, where many thousands of genes can be analyzed in parallel.

DNA chips generally consist of thin wafers of glass, silicon, or plastic having numerous microscopic bits of bio-molecules or porous support medium containing bio-molecules, such as immobilized DNA probe sequences arrayed on the surface. These are used to identify specific disease genes and to speed drug discovery efforts. It is anticipated that in the future increased use of bio-molecule related science will allow for a more personalized practice of medicine, more particularly the design and use of customized treatments and therapies based on a patient's genetic makeup.

Currently, bio-chips, more specifically DNA chips, are known that are based on a common method of manufacture, namely the etching of silicon computer chips, as currently utilized in the semiconductor industry. Of all of the uses of bio-chips to study bio-molecules, the study of DNA is the most mature. In one specific instance, a photoactivated DNA probe synthesis process is used to manufacture high density DNA chips (Affymetrix Corp.). Typically 80 photolithographic mask levels are used to synthesize 20mer DNA probes. An alternative method is to use dispensing techniques to place purified, presynthesized oligonucleotides onto specific locations on a surface to produce a DNA chip. The latter technique does not require photolithography and requires fewer redundant probes because the purity of the probe sequences is much higher than in the photoactivated probe synthesis process. An alternative means for synthesizing DNA probes is by using tiny micromirrors which allow for the placement of in excess of 300,000 bits of DNA onto a chip in just a few hours. In addition, the use of ink-jet printing is known, using high-speed robotic devices to print DNA on tiny squares of glass, to form an array. These types of machines are capable of forming as many as 32,000 DNA molecules on a single chip.

Still other methods include the use of fiber-optic bundles to build chips capable of holding 50,000 different DNA fragments on a single chip, and microelectronic chips that utilize electricity to attach DNA molecules to the surface of the chip.

For many types of applications, there is a need to define very small amounts of fluid (liquid) on a surface. Currently, this is done by dropping a small amount of liquid through an orifice by using some pressure to drop the liquid itself. Piezoelectric force may be applied to dispense small amounts (picoliters to nanoliters) of liquid. Although in some instances, the piezoelectric force may result in undesired changes in the fluid properties and the denaturing of the bio-molecules contained in the fluid. Some applications need the dispensing of drops of a large number of different liquids. The time needed to complete the fill, dispense, and wash cycles of piezo-dispense tips for each different liquid is prohibitively long in any situation where the dispense of tens, thousands or tens of thousands of different liquids are needed. In some applications, the amount of liquid needed may be even smaller.

Accordingly, it is a purpose of the present invention to provide for an apparatus and method for depositing a small controlled amount of liquid onto a target.

SUMMARY OF THE INVENTION

In accordance with the objects outlined above, the present invention provides devices comprising fluid reservoirs comprising at least one (and preferably a plurality) of holding wells and dipping wells, interconnected with microfluidic channels to be in fluid communication. Optionally, the holding and dipping wells and channels are integrally formed with a semiconductor material. Optionally, the holding wells are formed about the perimeter of a silicon wafer, with the dipping wells in a central

aspect. The dipping wells may be formed in either a two-dimensional array or formed within the silicon wafer at a plurality of planar levels. The holding wells can be characterized as holding a known volume of a liquid of a certain viscosity and the dipping wells can be characterized as holding the known volume of a liquid of a certain viscosity. The channels can be characterized as transporting the known volume of a liquid of a certain viscosity from the at least one holding well to the at least one dipping well, the at least one microfluidic channel extending from the at least one holding well to the at least one dipping well, thereby placing the holding well and the dipping well in fluidic communication.

The fluid reservoir components can be formed by selective etching or wet etching. In one aspect, the invention provides methods of fabricating a fluid reservoir for use in a bio-array fabricating system comprising the steps of providing a semiconductor material, forming an oxide on an uppermost surface of the semiconductor material; forming an aluminum material on an uppermost surface of the oxide in an area to define at least one microfluidic channel; forming an oxide material on an uppermost surface of the aluminum material; depositing a mask on an uppermost surface of the oxide material; etching the oxide material to define at least one holding well and at least one dipping well having a defined shape, each of the at least one holding well and the at least one dipping well characterized as holding a known volume of a liquid of a certain viscosity; and etching the aluminum material to define the at least one microfluidic channel in fluidic communication with the at least one holding well and the at least one dipping well, the at least one microfluidic channel characterized as transporting the known volume of a liquid from the at least one holding well to the at least one dipping well. Optionally, the methods include the step of utilizing chemical mechanical polishing (CMP) to remove excess aluminum material thereby leaving aluminum material in the etched channel prior to forming the oxide material on the uppermost surface of the aluminum.

In a further aspect, the invention provides stamps comprising a base portion; and a plurality of tips integrally formed with and protruding from the base portion, the plurality of tips characterized as holding a known volume of a liquid of a certain viscosity under surface tension. The tips can be optionally formed in a two-dimensional array, and can be formed by selective etching, or formed to define a hydrophilic and hydrophobic structure using isotropic etch techniques. Optionally, the plurality of tips are formed including a depth stop using anisotropic etch techniques (or other techniques) for the control of the level of immersion of the plurality of tips. The tips can be formed having an oxide structure at a tip endpoint. The stamps may have 30,000-50,000 tips are formed in a 1 cm space.

In a further aspect, the invention provides methods of fabricating a stamp for controlled dispensing of a liquid comprising the steps of: providing a semiconductor material; depositing a mask on an uppermost surface of the semiconductor material; and etching the semiconductor material to define a plurality of tips each having a defined shape, each of the plurality of tips characterized as holding a known volume of a liquid of a certain viscosity under surface tension. Optionally, the methods include the step of depositing a thick oxide on an uppermost surface of the semiconductor material prior to

etching. The etching step can include etching the silicon material using isotropic etch techniques to form a structure defining a hydrophilic portion and a hydrophobic portion.

In an additional aspect, the invention provides bio-array fabricating system comprising a stamp including a plurality of tips formed thereon a surface; a fluid reservoir including a plurality of dipping wells each holding a sensing liquid formed complementary to the plurality of tips formed thereon a surface of the stamp, the fluid reservoir further including a plurality of microfluidic channels; and a sensor plate including a surface onto which a minute volume of the sensing liquid is deposited by the plurality of tips once dipped into the dipping wells containing the sensing liquid, thereby forming a bio-array on the surface of the sensor plate. The tips can be anisotropic etched to define a depth stop.

In a further aspect, the system including a detector, on or near the sensor plate, such as an optical or electronic detector.

In an additional aspect, the invention provides a bio-array fabricating system comprising a stamp formed of a silicon material and including a plurality of tips formed thereon a surface, the stamp further defining a depth stop; a fluid reservoir including a plurality of dipping wells each holding a sensing liquid formed complementary to the plurality of tips formed thereon a surface of the stamp, the fluid reservoir further including a plurality of microfluidic channels in fluidic communication with a plurality of holding wells; and a sensor plate including a surface onto which a minute volume of the sensing liquid is deposited by the plurality of tips once dipped into the dipping wells containing the sensing liquid, thereby forming a bio-array on the surface of the sensor plate.

In a further aspect, the invention provides methods of controlled dispensing of a liquid comprising the steps of providing a stamp formed of a silicon material and including a plurality of tips formed thereon a surface, the stamp further defining a depth stop; providing a fluid reservoir including a plurality of dipping wells each holding a sensing liquid formed complementary to the plurality of tips formed thereon a surface of the stamp, the fluid reservoir further including a plurality of microfluidic channels in fluidic communication with a plurality of holding wells; providing a sensor plate including a surface; depositing a minute volume of the sensing liquid onto the sensor plate by dipping the plurality of tips into the dipping wells containing the sensing liquid and stamping the surface of the sensor plate, thereby forming a bio-array on the surface of the sensor plate. The array can be formed in a single stamping process step, or multiple steps.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top view of a fluid reservoir, illustrating a plurality of dipping wells, holding wells and microfluidic channels formed therein according to the present invention;

FIGs. 2-5 illustrate simplified side views and a top view of the steps utilized during fabrication of a fluid

reservoir including fabrication of a microfluidic channel according to the present invention;

FIGs. 6-10 illustrate simplified side views of the steps utilized during fabrication of a second embodiment of a fluid reservoir including fabrication of a microfluidic channel according to the present invention;

FIGs. 11-13 illustrate simplified side views of the steps utilized during fabrication of a channel void according to the present invention; and

FIG. 14 illustrates in simplified sectional view a multi-layered fluid reservoir chip according to the present invention.

Figure 15 is a simplified side view illustrating the stamp including a plurality of tips according to the present invention.

FIGs. 16-19 illustrate simplified side views of the steps utilized during fabrication of a stamp including a plurality of tips according to the present invention.

Figure 20 is a simplified side view illustrating a stamp and a reservoir of the bio-array fabricating system according to the present invention;

FIG. 21 is a simplified side view illustrating the stamp and sensor area of the bio-array fabricating system according to the present invention;

FIG. 22 is an enlarged side view illustrating a plurality of tips forming the stamp of the bio-array fabricating system according to the present invention;

FIG. 23 is an enlarged side view illustrating a dipping well formed in the fluid reservoir of the bio-array fabricating system according to the present invention;

FIG. 24 is a top view of the fluid reservoir, illustrating the microfluidic channels formed therein of the bio-array fabricating system according to the present invention; and

FIG. 25 is a top view of a sensor plate, illustrating the plurality of bio-array sites according to the present invention.

Figure 26 depicts a "one component" system as described herein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an apparatus and a method of dispensing a small quantity of a reactive liquid on a surface to produce a biochip. More particularly, disclosed is an apparatus and method for forming an array of sensing liquid on a surface for use in diagnostic assays. This depositing of a small controlled amount of liquid provides for the fabrication of a chip, such as a DNA chip, with numerous test sites, anticipated any where in excess of 100 test sites up to a million test sites, that remains cost and time effective in the manufacture of the chip.

In general, the present invention provides several different embodiments to accomplish the rapid and inexpensive formation of biological arrays. In a preferred embodiment, the system comprises a "one component" system, comprising a dispenser substrate that has a number of reservoir wells on one side, connected via offset microfluidic channels to dispensing heads on the opposite surface of the substrate. By controlling the size of the reservoir wells, the viscosity and surface tension of the reagent in the well, and the length and diameter of the offset channel, the fluid can be dispensed easily to a variety of sensor plates based on capillary action and surface tension.

Alternatively, the invention provides a "two component" system, with a first component comprising a substrate comprising a fluid reservoir. The fluid reservoir has holding wells, serving as reservoirs, and dipping wells, connected by channels. The second component comprising a stamping substrate that has dispensing tips that will fit into the dipping wells. Thus, by dipping the stamp into the fluid reservoir and contacting it with the sensor plate, arrays of reagents can be made.

Thus, the present invention provides methods and compositions for the formation of arrays of biological reagents. As will be appreciated by those in the art, the biological reagents can be a wide variety of different materials. In a preferred embodiment, the biological reagents are nucleic acids or proteins.

By "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramide (Beaucage et al., Tetrahedron 49(10):1925 (1993) and references therein; Letsinger, J. Org. Chem. 35:3800 (1970); Sprinzl et al., Eur. J. Biochem. 81:579 (1977); Letsinger et al., Nucl. Acids Res. 14:3487 (1986); Sawai et al, Chem. Lett. 805 (1984), Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); and Pauwels et al., Chemica Scripta 26:141 91986)), phosphorothioate (Mag et al., Nucleic Acids Res. 19:1437 (1991); and U.S. Patent No. 5,644,048), phosphorodithioate (Briu et al., J. Am. Chem. Soc. 111:2321 (1989), O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press), and peptide nucleic acid backbones and linkages (see Egholm, J. Am. Chem. Soc. 114:1895 (1992); Meier et al., Chem. Int. Ed. Engl. 31:1008 (1992); Nielsen, Nature, 365:566 (1993); Carlsson et al., Nature 380:207 (1996), all of which are incorporated

by reference). Other analog nucleic acids include those with positive backbones (Denpcy et al., Proc. Natl. Acad. Sci. USA 92:6097 (1995); non-ionic backbones (U.S. Patent Nos. 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Kiedrowshi et al., Angew. Chem. Intl. Ed. English 30:423 (1991); Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); Letsinger et al., Nucleoside & Nucleotide 13:1597 (1994); Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker et al., Bioorganic & Medicinal Chem. Lett. 4:395 (1994); Jeffs et al., J. Biomolecular NMR 34:17 (1994); Tetrahedron Lett. 37:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see Jenkins et al., Chem. Soc. Rev. (1995) pp169-176). Several nucleic acid analogs are described in Rawls, C & E News June 2, 1997 page 35. Nucleic acid analogs also include "locked nucleic acids". All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of electron transfer moieties, or to increase the stability and half-life of such molecules in physiological environments.

As will be appreciated by those in the art, all of these nucleic acid analogs may find use in the present invention. In addition, mixtures of naturally occurring nucleic acids and analogs can be made; for example, at the site of conductive oligomer or electron transfer moiety attachment, an analog structure may be used. Alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made.

As outlined herein, the nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xathanine hypoxathanine, isocytosine, isoguanine, etc. As used herein, the term "nucleoside" includes nucleotides and nucleoside and nucleotide analogs, and modified nucleosides such as amino modified nucleosides. In addition, "nucleoside" includes non-naturally occuring analog structures. Thus for example the individual units of a peptide nucleic acid, each containing a base, are referred to herein as nucleosides.

By "proteins" or grammatical equivalents herein is meant proteins, oligopeptides and peptides, derivatives and analogs, including proteins containing non-naturally occurring amino acids and amino acid analogs, and peptidomimetic structures. The side chains may be in either the (R) or the (S) configuration. In a preferred embodiment, the amino acids are in the (S) or L-configuration.

In addition, depending on the reaction and array, the biological reagent can be a completed

biomolecule, or a subunit that is added during synthesis. For example, in the case of nucleic acids, the biological reagent may be a full length nucleic acid that is added to the sensor plate. Alternatively, the present invention can be used in the synthesis of arrays; for example, a first nucleotide can be added using conventional methods, with washing as needed, and then a second nucleotide added, etc., to create the array in a step-wise fashion.

Alternatively, although not normally classified as "biological", arrays of other materials such as organic chemical moieties can be made in the same manner.

Accordingly, in a preferred embodiment, the present invention comprises a "one component" liquid dispensing system comprising a first substrate. As will be appreciated by those in the art, the substrates outlined herein can be made from a wide variety of materials, including, but not limited to, silicon such as silicon wafers, silicon dioxide, silicon nitride, glass and fused silica, gallium arsenide, indium phosphide, aluminum, ceramics, polyimide, quartz, plastics, resins and polymers including polymethylmethacrylate, acrylics, polyethylene, polyethylene terepthalate, polycarbonate, polystyrene and other styrene copolymers, polypropylene, polytetrafluoroethylene, superalloys, zircaloy, steel, gold, silver, copper, tungsten, molybdeumn, tantalum, KOVAR, KEVLAR, KAPTON, MYLAR, brass, sapphire, etc. Preferred embodiments utilize silicon and ceramic materials, depending on the reagents utilized. As will be appreciated by those in the art, the material comprising the substrate should be compatible with the reagents outlined herein.

In this embodiment, as generally depicted in Figure 20, the substrate comprises two surfaces, generally outlined herein as opposite each other. The first surface comprises a plurality of reservoir wells. By "reservoir well" herein is meant a chamber for the retention of fluids as outlined herein. The wells can be a variety of sizes depending on the density of the array and the number of arrays to be made in a single run.

The second surface of the substrate comprises a plurality of dispensing heads (sometimes referred to herein as pens or tips). As will be appreciated by those in the art, these can be configured in a wide variety of ways and include a number of different geometries, as is known in the art. In general, regular X-Y spacing is done.

The reservoir wells and dispensing tips are connected via a microchannel, as depicted in Figure26. As will be appreciated by those in the art, this may be done in a number of configurations; what is important is that the combination of the size of the reservoir, the surface tension and viscosity of the fluid being dispensed, and other factors, allows the controlled dispensing of the fluid. Generally, as shown in Figure 26, the well and corresponding head are "offset", such that there is some distance between them. This is particularly true in the case where the device is used to formulate a high density array; the tips will be close together on one surface to achieve the high density, but the

reservoirs may be spread out on the other surface.

In a preferred embodiment, the one component system is made using two polished silicon wafers; one that has been undercut with microfluidic channels on one side and dispensing heads on the other, and another with the reservoirs. Alternatively, a single two sided silicon wafer can be used; the channels can be produced using a sacrificial layer of Al coated with oxide, which is then subsequently removed using wet etching.

Once fabricated, the reservoirs are filled using standard techniques and the dispenser is contacted with one or more sensor plates to form the array. As will be appreciated by those in the art, the sensor plate can comprise a wide variety of materials, including glass, ceramics, plastics, etc., as outlined above for substrates. In some embodiments, the sensor plate is coated or pretreated with materials such as chemical functionalities or hydrogels to allow the attachment (in some cases covalent attachment) of the biological reagent(s).

In an additional preferred embodiment, the present invention provides a two component system comprising a fluid reservoir and a stamp of tips. The fluid reservoir comprises a substrate, as outlined above, comprising at least one, and preferably a plurality of holding wells and dipping wells, each connected by a microfluidic channel.

A preferred embodiment is illustrated in Figures 1-14. FIG. 1 illustrates a fluid reservoir including a plurality of dipping wells, holding wells and microfluidic channels formed as a part thereof and FIGs. 2-11 illustrate the steps in fabricating a fluid reservoir according to the present invention.

Referring now to FIG. 1, illustrated is a top plan view of fluid reservoir 10 illustrating the layout of a plurality of dipping wells 12, a plurality of holding wells 14, and a plurality of microfluidic channels 16. Reservoir 14 is formed using standard semiconductor processing techniques. More specifically, reservoir 10 is formed including the plurality of wells 12 and 14, generally similar to the formation of a semiconductor chip including a plurality of fanned-out bonding pads. In particular, the plurality of microfluidic channels 16 are formed in fluidic communication with the plurality of holding wells 14, which serve as large liquid storage reservoirs, formed about the perimeter of fluid reservoir 10 and dipping wells 12, formed within a central aspect 11 of fluid reservoir 10, as denoted in FIG. 1 by a dashed line. Typically holding wells 14 are on the order of 100 microns while the actual dipping well 12 array dimension can be on the order of a few microns to 10's of microns.

Referring now to FIGs. 2-5, illustrated is a portion of fluid reservoir 10, showing steps in the fabrication of a first embodiment of fluid reservoir 10. As illustrated in FIG. 2, a semiconductor material, more particularly a silicon wafer, 20 is provided having had an uppermost surface 21 cleaned prior to further deposition of materials. It should be understood that it is anticipated by this disclosure that any type of

semiconductor material, such as plastic, glass, or the like can be used to form fluid reservoir 10. Surface 21 has formed thereon, a layer of an oxide 22, such as silicon dioxide. A layer of an aluminum material 24 is next deposited in an area that will form dipping wells 12, holding wells 14, and channels 16. It should be understood that any other material that can be selectively etched away, such as any metal or insulative material, can be utilized as material 24. Next, a layer of a thick oxide or nitride material, 26 is grown over the surface of silicon material 20 and aluminum material 24, thereby making the surface inert to fluids being held or transferred through wells 12 and 14 and channels 16.

Referring now to FIG. 3, illustrated in a side sectional view, taken along an opposing direction from FIG. 2, is a portion of fluid reservoir 10. As illustrated, a mask layer 28, such as a photoresist, is deposited on top of oxide layer 26. Mask layer 28 provides for etching of oxide layer 26 to define holding wells 14 and dipping wells 12 as illustrated in FIG. 4. During fabrication, oxide material 26 is etched, as illustrated in FIG. 4, to expose a portion of aluminum material 24. Next, aluminum material 24 is wet etched to define a flow-through microfluidic channel 26, placing holding well 14 and dipping well 12 in fluidic communication.

Referring now to FIG. 5, illustrated in a simplified top view is a portion of fluid reservoir 10, more particularly illustrated is a reservoir chip including one holding well 14, one dipping well 12, and a microfluidic channel 16, illustrated by a dashed line. It should be understood that in the present embodiment, fluid reservoir 10 includes a plurality of holding wells 14, dipping wells 12 and microfluidic channels 16.

Referring now to FIGs. 6-10, illustrated is a portion of fluid reservoir 10', showing steps in the fabrication of a second embodiment of fluid reservoir 10'. It should be noted that all components similar to the components illustrated in FIGs. 2-5, are designated with similar numbers, having a prime added to indicate the different embodiment. As illustrated in FIG. 6, a silicon wafer 20' is provided having had an uppermost surface 21' cleaned prior to further deposition of materials. Silicon wafer 20' has etched therein a channel 25. Surface 21' has formed thereon, including within channel 25, a layer of an oxide 22', such as a silicon dioxide.

Referring now to FIG. 7, a layer of an aluminum material 24' is next deposited on surface 21', including and within channel 25, so as to deposit aluminum material 24' in all areas that will form dipping wells 12', holding wells 14', and channels 16' (discussed presently). Next, aluminum material 24' is planarized using a chemical mechanical polishing (CMP) technique to leave material 24' deposited within channel 25. As illustrated in FIG. 8, next a layer of a thick oxide, or nitride material, 26' is grown over the surface of silicon material 20' and aluminum material 24', thereby making the surface inert to fluids being held or transferred through wells 12' and 14' and channels 16'.

Referring now to FIG. 9, illustrated in a side sectional view, taken along an opposing direction from

FIGs. 6 and 7, is reservoir chip 10'. As illustrated, a mask layer 28', such as a photoresist, is deposited on top of oxide layer 26'. Mask layer 28' provides for etching of oxide layer 26' to define holding wells 14' and dipping wells 12' as illustrated in FIG. 10. During fabrication, oxide material 26' is etched, as illustrated in FIG. 10, to expose aluminum material 24'. Next, aluminum material 24' is wet etched to define microfluidic channel 26', placing holding well 14' and dipping well 12' in fluid communication.

Referring now to FIGs. 11-13, illustrated in simplified sectional views, are steps in the method of fabricating reservoir chip 10 when a long channel 16 is required, thus making wet etching of the aluminum material 24 contained within the channel difficult. In this instance, a void 30 is etched into oxide 26 along a length of channel 16 containing aluminum material 24 to gain access to aluminum material 24. As illustrated in FIG. 12, aluminum material 24 is wet etched away, leaving channel 16 for microfluidic communication. Next, as illustrated in FIG. 13, an oxide material 32, or other type of material that is not reactive with DNA, is grown or deposited on the surface of oxide material 26 using either angular evaporation, or angular deposition. This angular depositing or evaporating technique allows for the filling of void 30 without any oxide material 32 reaching the channel 16 formed therein oxide material 26.

In the preferred embodiments, holding wells 14 supply a fluid to dipping wells 12 through microfluidic channels 16. To accomplish such feeding of a fluid through channels 16, it is disclosed that capillary action can be utilized to feed the fluid, a vacuum can be used on the wells to transfer the fluid from the holding wells to the dipping wells, or pressure at the holding wells can be used.

Referring now to FIG. 14, illustrated in simplified side sectional view is a portion of fluid reservoir chip 10 showing a plurality of holding wells 14, and dipping wells 12, having formed therebetween so as to place holding wells 14 and dipping wells 12 in fluidic communication, a plurality of microfluidic channels 16. In this particular embodiment, there is illustrated a plurality of planar levels of formation of channels 16 within the silicon wafer 20. This plurality of levels, provides for the decrease in overall dimension of the fluid reservoir 10. In a preferred embodiment fluid reservoir 10 in a preferred embodiment has an overall dimension of approximately 2mm square. A central aspect having a dimension of approximately 200μ square has located therein dipping wells 12. Accordingly, when a plurality of planar levels for fabricating channels 16 are utilized, a dimension of less than 2mm square can be implemented for fluid reservoir 10.

During use of a bio-array fabricating system, in addition to fluid reservoir 10, a two-dimensional array of tips is formed of a silicon material of a required size and shape using selective etch techniques. The tips when dipped in a liquid held within dipping wells 12, such as a sensing liquid, typically of the type utilized in DNA research/diagnosing, hold a small sample of the liquid due to surface tension. Further information on the use of a stamp and a bio-array fabricating system, can be found in co-

pending patent application, entitled "BIO-ARRAY FABRICATING SYSTEM AND METHOD FOR CONTROLLED DISPENSING OF A LIQUID", bearing attorney docket number CR 99-030, filed simultaneously herewith, assigned to the same assignee and incorporated herein by this reference and co-pending patent application, entitled "STAMP WITH SILICON TIPS FOR CONTROLLED DISPENSING OF A LIQUID AND METHOD OF FABRICATION", bearing attorney docket number CR 99-029, filed simultaneously herewith, assigned to the same assignee and incorporated herein by this reference.

Thus, a fluid reservoir, or reservoir chip, including a plurality of holding wells, dipping wells and microfluidic channels for use in a bio-array fabricating system in which controlled dispensing of a liquid is sought, is disclosed. As disclosed, this fluid reservoir utilizes well known techniques of silicon processing. The fluid reservoir is anticipated for use in the area of protein and DNA hybridization array formation. Accordingly, such instances are intended to be covered by this disclosure.

Additional preferred embodiments are depicted in Figures 15-19. More particularly, illustrated in FIG. 15, is a side view illustrating a portion of a big-array fabricating system, more specif-cally, illustrated is a stamp, generally referenced 10, and including a plurality of tips 12 formed as a part thereof. As illustrated, stamp 10 includes a plurality of spaced apart tips 12 formed utilizing semiconductor manufacturing techniques. More particularly, in this particular embodiment, tips 12 are formed using isotropic and anisotropic etching techniques (discussed presently). Stamp 10, including tips 12, are preferably formed of a silicon material. During fabrication, tips 12 are formed by depositing a thick layer of an oxide or nitride and a mask, such as a photoresist, and wet and dry etching under predetermined conditions that will enable formation of tips 12 with differing, yet controlled, tip edges and shapes. Tips 12, as will be discussed presently, when dipped in a fluid of a certain viscosity can hold a small volume of liquid, more particularly a sensing liquid (as illustrated in FIG. 5), as a result of surface tension.

Referring now to FIGS. 16-19, illustrated are the various steps required in the manufacturing of a stamp with silicon tips for controlled dispensing of a liquid. More particularly, illustrated is a portion of stamp 10, illustrating the fabrication of a single tip 12 included as a part of the plurality of tips 12 formed as a part of stamp 10. As previously stated, a two-dimensional array of tips 12 is formed of a silicon material of a required size and shape using selective etch techniques. Tips 12 when dipped in a liquid, such as a sensing liquid, typically of the type utilized in DNA research/diagnosing, hold a small sample of the liquid due to surface tension.

Referring again to FIGs. 16-19, illustrated in an enlarged sectional side view is a portion of stamp 10, illustrating fabrication of a single tip 12. As illustrated, tip 12 is formed using isotropic and anisotropic etch techniques. Stamp 10 (as illustrated in FIG. 15), more specifically is formed of a base portion 14 having protruding therefrom tips 12. As illustrated in FIG. 16, the first step in the fabrication of stamp

10 is to provide a silicon material forming base portion 14. Base portion 14 includes a silicon material 16 having formed thereon an uppermost surface 17, a silicon oxide 18 and a mask, such as a photoresist, 20.

Referring now to FIG. 17, silicon oxide 18 is selectively etched down to silicon material 16 as shown and photoresist or mask 20 is removed subsequent to the selective etching. As illustrated in FIG. 18, isotropic etching is utilized to form an angled portion 22, thus forming a pointed structure, and anisotropic etching is utilized, as illustrated in FIG. 19, to form perpendicular sides 24, thereby providing for a depth stop (discussed presently) and allowing for a smaller pitch between tips 12. This series of selective etch techniques provides for tip 12 to have a well defined shape and size using conventional materials and will also allow for ease of integration with other electronics. In a preferred embodiment, tips 12 are spaced having a pitch ranging from 5um-1 mm, with a preferred pitch of 30 um or less. This pitch spacing provides for the placement of 30,000-50,000 tips 12 in a 1 x 1 cm space.

In this particular embodiment, each tip 12 is formed having side 24 that is substantially perpendicular to base portion 14 and angled portion 22, that comes to a point defining end points 28 of tips 12. Tips 12 are formed to provide for the correct adherence of a sensing liquid. In an alternative embodiment, silicon oxide 18 is left remaining on endpoint 28 and thus provides for an increased area in which the sensing liquid adheres to. It should be understood that while silicon oxide 18 is specifically named, alternative oxides, nitrides or metals can be used. This material left remaining on endpoint 28 will allow for the definition of hydrophilic and hydrophobic portions or areas, suitable for use with certain liquids. For example, in order to get better control on the volume of DNA containing liquid adhered to end points 28 of tips 12, angled portion 22, side 24, and surface 30 can be treated to obtain hydrophobic portions.

In this particular embodiment, during the fabrication process tips 12 are formed having a specific height "h" as illustrated in FIG. 19, measured from a surface 30 of base portion 14 to the end point 28 of tip 12. This specific depth measurement provides for a stopping point when stamp 10 is dipped into the sensing liquid typically contained in complementary formed well structures, referred to as dipping wells. As illustrated in FIG. 19, sensing liquid 32 adheres to end points 28 of tips 12 due to surface tension. This depth measurement provides for the proper "dipping" of the endpoints 28 of tips 12 within sensing liquid 32. It should be understood that as illustrated, remaining silicon oxide 18 has been removed from endpoint 28.

Alternatively, and as illustrated in FIG. 15, surface 26 of stamp 10, provides for a stopping point when stamp 10 is dipped into the sensing liquid typically contained in complementary fonned well structures. More particularly, surface 26 of stamp 10, meets Nvith a surface of a fluid reservoir chip including a plurality of dipping wells, and provides for proper placement of end point 28 into the sensing liquid.

During operation, stamp 10 is dipped into the complementary formed fluid reservoir, more particularly the dipping wells, and properly positioned due to the abutment of surface 26 of stamp 10 and a surface of the reservoir chip, or some other similar depth stop. It should be understood that although a specific type of stopping method is illustrated in the figures which allows for proper positioning of stamp 10 relative to the reservoir chip, alternative means for positioning stamp 10 relative to the reservoir chip are anticipated by this disclosure, such as a protrusion from stamp 10 that rests on a recessed portion of the reservoir chip, or the like. Next, sensing liquid 32 is adhered to end point 28 of each tip 12 dependent upon the size and shape of tip 12. Once a prescribed amount of sensing liquid 32 is adhered to tips 12, stamp 10 is positioned relative to a sensor plate (not shown). The sensor plate, once stamped provides for multiple test sites. It should be understood to attain the sought after density of test sites on a surface of the sensor plate, that multiple stamping actions can be utilized. Thus, a stamp with silicon tips for controlled dispensing of a liquid and method of fabricating the stamp for use in a big-array fabricating system are disclosed. As disclosed, this system utilizes well known techniques of silicon processing. The stamp is anticipated for use in the area of big-array formation for big-molecule conjugation events such as protein and DNA hybridization array formation. Accordingly, such instances are intended to be covered by this disclosure.

A further preferred embodiment is shown in Figures 20-25. FIGs. 20-24 illustrate the plurality of components which compose the bio-array fabricating system and method of using the bio-array fabricating system for controlled dispensing of a liquid according to the present invention. More particularly, illustrated in FIG.20, is a side view illustrating a portion of a bio-array fabricating system, generally referenced 10, and including a stamp 12 and a fluid reservoir 14. Stamp 12 is composed of a plurality of tips 16 (discussed presently). Fluid reservoir 14 includes a plurality of dipping wells 18 (discussed presently) and a plurality of holding wells (not shown) formed therein, for the "holding" of a sensing liquid 20. It should be understood that dipping wells 18 are "fed" the sensing liquid through a plurality of microfluidic channels (not shown) formed therein fluid reservoir 14 connecting dipping wells 18 with the holding wells.

As illustrated, stamp 12 includes a plurality of spaced apart tips 16 formed utilizing semiconductor manufacturing techniques. More particularly, in this particular embodiment, tips 16 are formed using isotropic and anisotropic etching techniques. Stamp 12, and more specifically tips 16, are formed of silicon. During fabrication, tips 16 are formed by depositing an oxide or nitride mask and wet and dry etching under predetermined conditions that will enable formation of tips 16 with differing, yet controlled, tip edges and shapes. Tips 16, as will be discussed presently, when dipped in a fluid of a certain viscosity can hold a small volume of liquid, more particularly sensing liquid 20, as a result of surface tension.

In general, a two-dimensional array of tips 16 is formed of silicon of a required size and shape using selective etch techniques. Tips 16 when dipped in a liquid, such as sensing liquid 20 typically of the

type utilized in DNA research/diagnosing, hold a small sample of the liquid due to surface tension.

Referring now to fluid reservoir 14, fluid reservoir 14 as previously stated is formed to include plurality of dipping wells 18 and a plurality of holding wells (not shown). Dipping wells 18 are formed to hold sensing liquid 20 and are fed through a plurality of microfluidic channels 22 (discussed presently). During use, tips 16 are dipped into dipping wells 18, which hold sensing liquid 20 dependent upon use. To obtain varying sensing, or sample, liquids on different tips 16, dipping wells 18 into which each tip is dipped into must contain the correct liquid 20. This can be achieved by forming an array of small wells, which can hold the different liquids. Fluid reservoir 14 is formed complementary to stamp 12, as is illustrated by the spacing of tips 16 and dipping wells 18.

Referring now to FIG. 21, illustrated in simplified diagram is stamp 12 and sensor area, or sensor plate, 30. Sensor plate 30 is defined as the material onto which the bio-array pattern is to be formed. During fabrication of the bio-array sensor, stamp 12, having thousands of tips 16 formed therein, is aligned to reservoir 14 to pick up sensing liquid 20, as previously described, and then stamp 12 is aligned with sensor plate 30 to simultaneously transfer thousands of different sensing fluids in one stamping step to sensor plate 30.

Referring now to FIG. 22, illustrated in an enlarged sectional side view is a portion of stamp 12, including tips 16. As illustrated, stamp 16 is formed using isotropic and anisotropic etch techniques to form tips 16. Stamp 12, more specifically is formed of a base portion 32 having protruding therefrom tips 16. During fabrication tips 16 are formed having a specific height "h" as illustrated, measured from a surface 34 of base portion 32 to the end point of tip 16. As illustrated in FIG. 3, sensing liquid 20 adheres to end points 36 of tips 16 due to surface tension.

In this particular embodiment, each tip 16 is formed having a side 38 that is substantially perpendicular to base portion 32 and an angled end portion 40, that comes to a point defining end points 36 of tips 16. Tips 16 are formed to provide for the correct adherence of sensing liquid 20.

Referring now to FIGs. 23 and 24, illustrated is a side sectional view of a portion of fluid reservoir 14 showing formation of a dipping well 18 and a top plan view of fluid reservoir 14 illustrating the layout of dipping wells 18, an a plurality of microfluidic channels 22 (discussed presently). Fluid reservoir 14 is formed using standard semiconductor processing techniques. More specifically, as illustrated in FIG. 24, fluid reservoir 14 is formed including the plurality of dipping wells 18, generally similar to the formation of a semiconductor chip that includes a plurality of fanned-out bonding pads. In particular, the plurality of microfluidic channels 22 are formed in fluidic communication with a plurality of large liquid storage reservoirs 50, or holding wells, formed about the perimeter of reservoir 14. Typically holding wells 50 are on the order of 100 microns while the actual array dimension can be on the order of a few 10's of microns.

As illustrated in FIG. 24, fluid reservoir 14, more particularly dipping wells 18, are formed having a depth of "h". Depth "h" is complementary to depth "h" of stamp 12, as previously discussed. This same depth measurement provides for the proper "dipping" of the endpoints 36 of tips 16 within the sensing liquid 20. In addition, surface 52 of fluid reservoir 14, provides for a stopping point when stamp 12 is dipped into dipping wells 18. More particularly, surface 34 of stamp 12, meets with surface 52 of fluid reservoir 14, and provides for proper placement of end point 36 into sensing liquid 20. As illustrated sensing liquid 20 is fed into dipping wells 18 through microfluidic channels 22. It should be understood that in this particular embodiment, holding wells 50 may serve a single dipping well 18, or a plurality of dipping wells 18, and that it is anticipated by this disclosure that a greater number of holding wells 50 can be provided so that each dipping well 18 can hold a different sensing liquid 20 dependent upon the specific application for the device.

During operation, stamp 12 is dipped into reservoir 14, more particularly dipping wells 18, and properly positioned due to the abutment of surface 34 of stamp 12 and surface 52 of reservoir 14. It should be understood that although a specific type of stopping method is illustrated in the figures which allows for proper positioning of stamp 12 relative to reservoir 14, alternative means for positioning stamp 12 relative to fluid reservoir 14 are anticipated by this disclosure, such as a protrusion from stamp 12 that rests on a recessed portion of reservoir 14, or the like. Next, sensing liquid 20 is adhered to each tip 16. Once a prescribed amount of sensing liquid 20 is adhered to tips 16, stamp 12 is positioned relative to sensor plate 30. As illustrated in FIG. 6, sensor plate 30, once stamped provides for multiple test sites 60. It should be understood to attain the sought after density of test sites 60 on a surface 61 of sensor plate 30, that multiple stamping actions can be utilized.

Thus, a system for the fabrication of a bio-array and a method of dispensing a controlled amount of liquid onto a surface thereby forming the bio-array are disclosed. As disclosed, this system utilizes well known techniques of silicon processing. The system is anticipated for use in the area of protein, RNA and DNA hybridization array formation. Accordingly, such instances are intended to be covered by this disclosure.

All references cited herein are incorporated by reference.

CLAIMS

We claim:

1. A device comprising a fluid reservoir comprising a first substrate comprising:
   a) at least one holding well;
   b) at least one dipping well; and
   c) at least one microfluidic channel connecting said holding well and said dipping well.

2. A device according to claim 1 wherein said fluid reservoir comprises:
   a) a plurality of holding wells;
   b) a plurality of dipping wells; and
   c) a plurality of microfluidic channels, each connecting a holding and dipping well.

3. A device according to claim 1 or 2 further comprising a stamp comprising a second substrate comprising a plurality of dispensing tips.

4. A device according to claim 1, 2 or 3 wherein said substrate comprises a semiconductor material.

5. A dispenser for liquid dispensing comprising a substrate comprising a first and a second surface, said first surface comprising a plurality of reservoir wells, said second surface comprising a plurality of dispensing heads, and said substrate further comprising a plurality of microfluidic channels, each connecting a reservoir well with a dispensing head.

6. A method of making a bioarray comprising:
   a) providing a fluid reservoir comprising:
      i) a plurality of holding wells;
      ii) a plurality of dipping wells; and
      iii) a plurality of microfluidic channels, each connecting a holding well with a dipping well;
   wherein said holding wells, said dipping wells and said microfluidic channels contain a fluid comprising a biological reagent;
   b) providing a stamp comprising a first substrate comprising a plurality of dispensing tips;
   c) contacting said dispensing tips into said dipping wells to load said tips with said biological reagent; and
   d) contacting said tips with a sensor plate to form a bioarray.

7. A method of making a bioarray comprising:
   a) providing a dispenser comprising a substrate comprising a first and a second

surface, said first surface comprising a plurality of reservoir wells, said second surface comprising a plurality of dispensing heads, and said substrate further comprising a plurality of microfluidic channels, each connecting a reservoir well with a dispensing head, wherein said reservoir wells, said dispensing heads, and said channels contain a fluid comprising a biological reagent; and

b) contacting said dispensing heads with a sensor plate to form a bioarray.

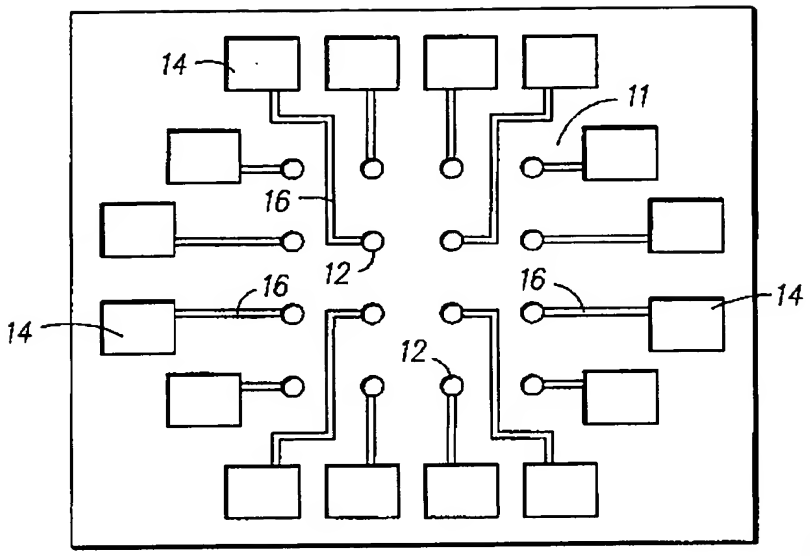

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FIG. 1

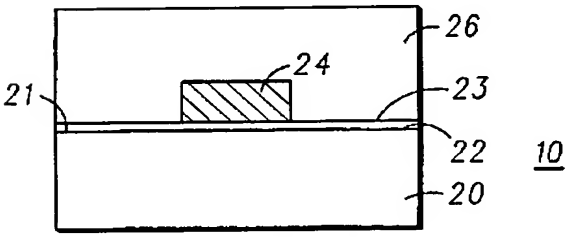

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FIG. 2

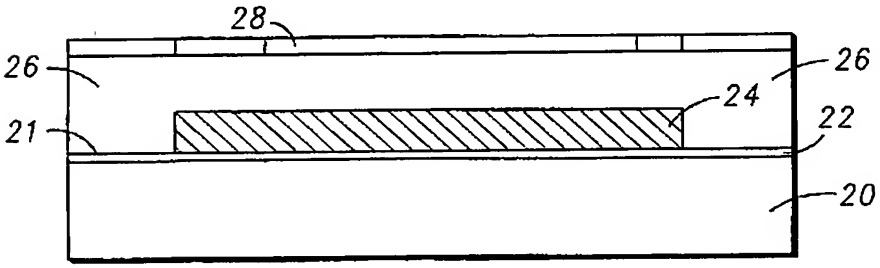

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FIG. 3

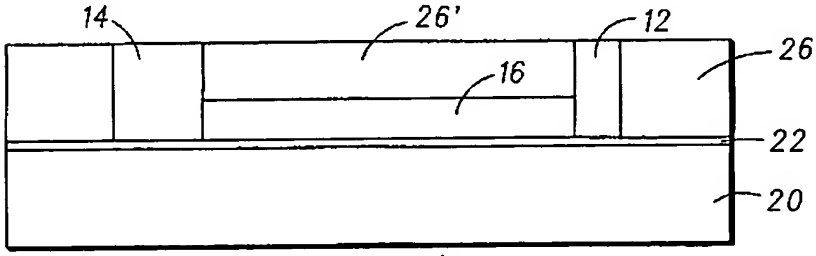

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FIG. 4

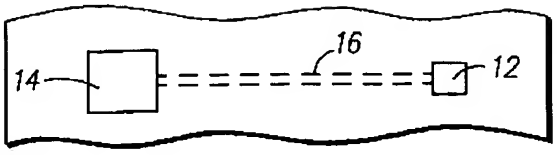

FIG. 5

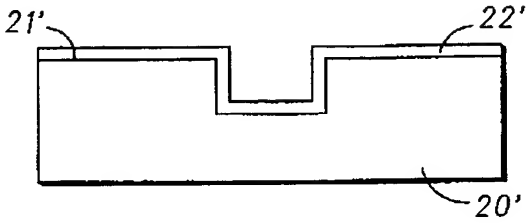

FIG. 6

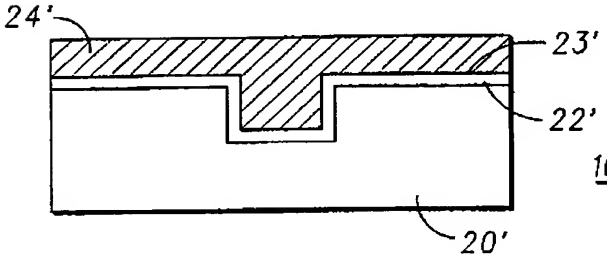

FIG. 7

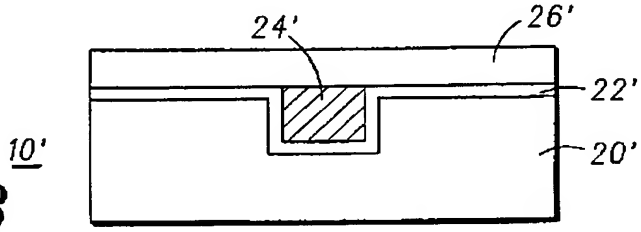

FIG. 8

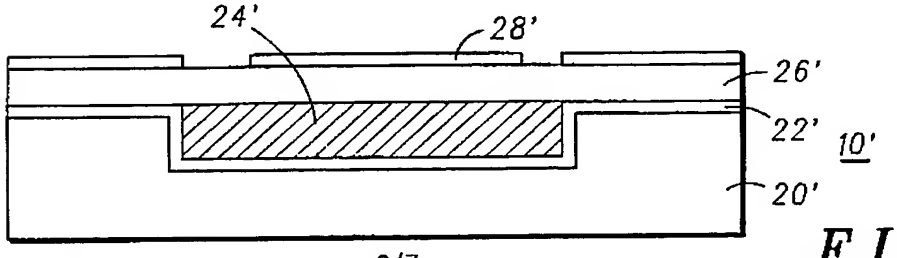

FIG. 9

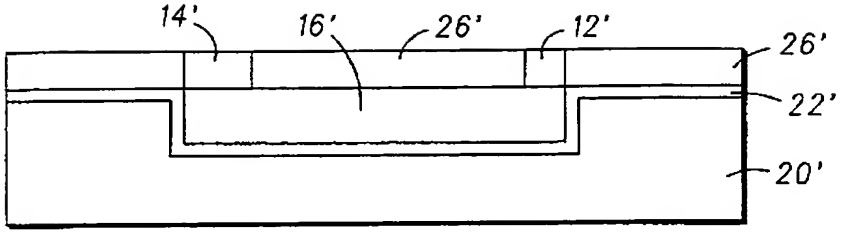

FIG. 10

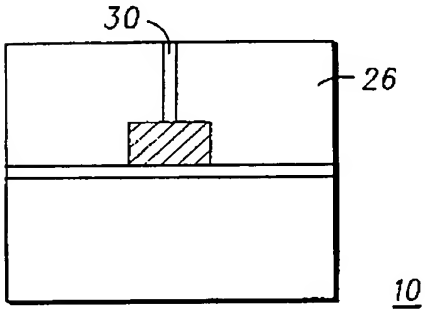

FIG. 11

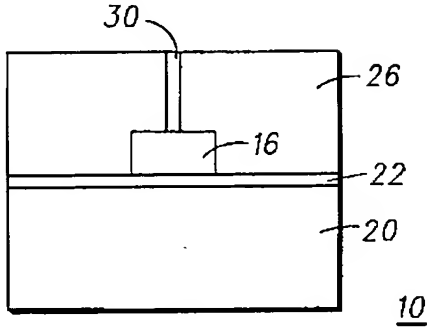

FIG. 12

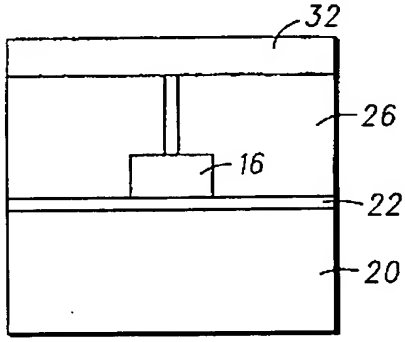

FIG. 13

FIG. 14

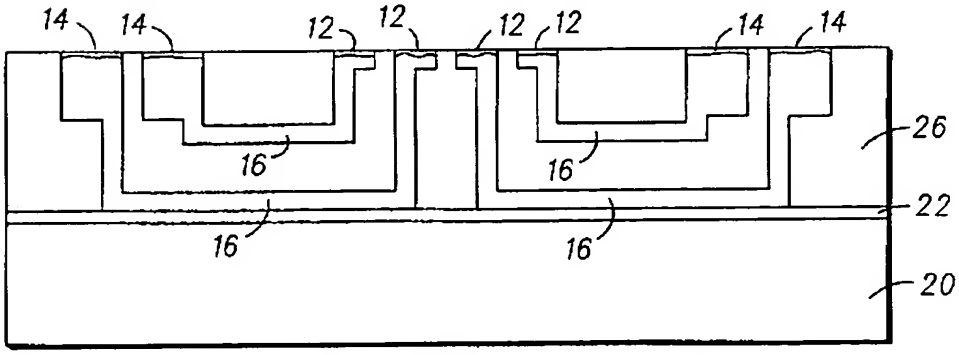

FIG. 15

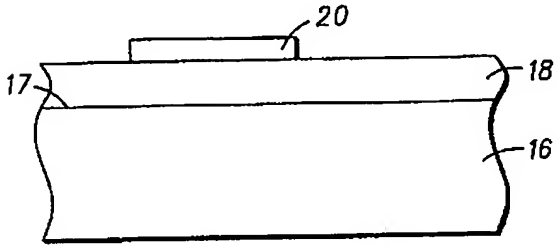

FIG. 2-16

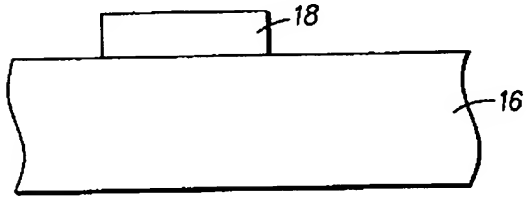

FIG. 3-17

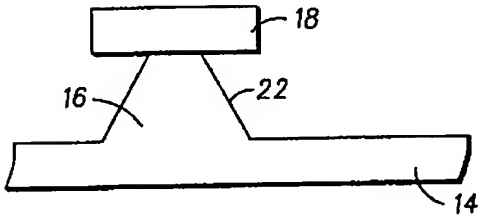

FIG. 4-18

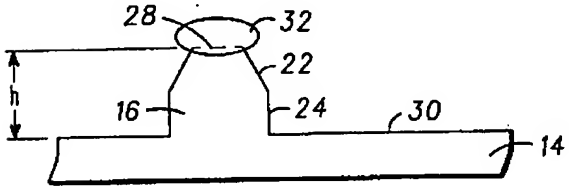

FIG. 5-19

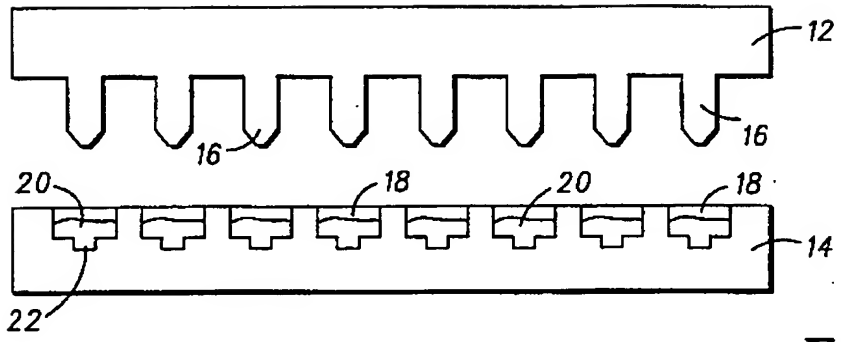

FIG. 1 20

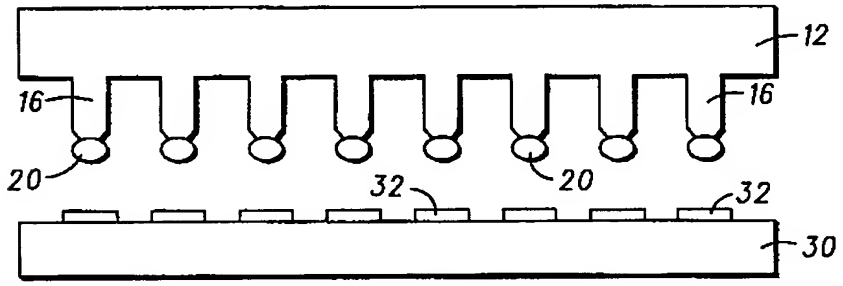

FIG. 2 1

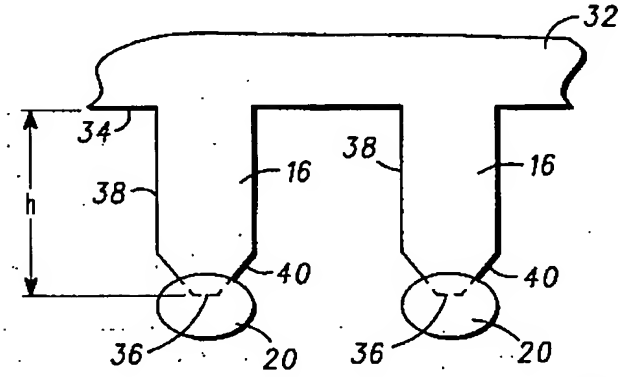

FIG. 3 22

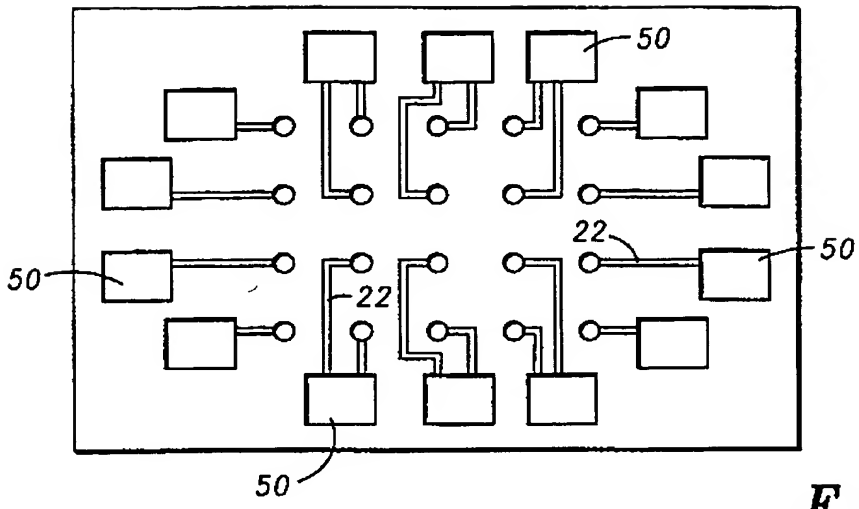

FIG. 4-23

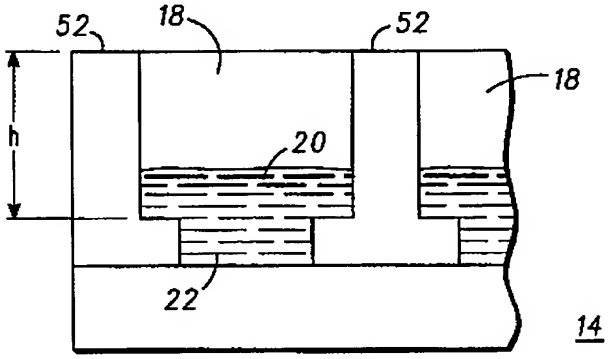

FIG. 5-24

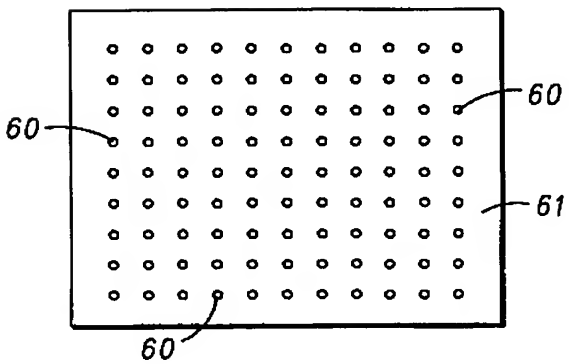

FIG. 6-25

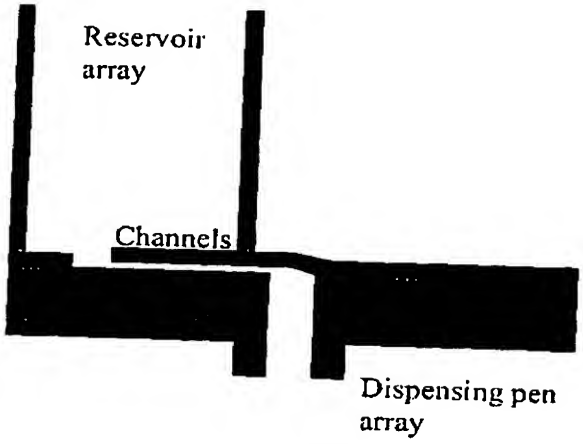

Fig 26

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/34361

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 B01L3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, INSPEC, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 955 084 A (CORNING INC) 10 November 1999 (1999-11-10) abstract; figures 1,11,12,14-18 column 8, line 43 -column 9, line 19 column 11, line 7 -column 12, line 14 column 15, line 35 -column 17, line 22	1-7
A	US 5 882 930 A (BAIER JOERG) 16 March 1999 (1999-03-16) abstract; figures 1-9 column 1, line 39 -column 3, line 15 column 3, line 50 -column 6, line 23	1-7
A	WO 98 45693 A (SOANE DAVID S ;SOANE ZOYA M (US); ACLARA BIOSCIENCES (US); AMIGO M) 15 October 1998 (1998-10-15) abstract; figures 1,2,5,6 page 6, line 24 -page 7, line 20	1-4

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 2001	29/05/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Runser, C

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International Application No
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